Claims

1. Method for the manufacture of a nucleic acid molecule comprising the following steps:

a) providing a first at least partially double-stranded oligonucleotide, whereby the oligonucleotide comprises a first and a second single-stranded overhang,

b) providing a second at least partially double-stranded oligonucleotide, whereby the oligonucleotide comprises a recognition site for a first type IIS restriction enzyme which cuts outside its recognition site, a modification allowing the oligonucleotide to be coupled to a surface and a single-stranded overhang,

c) ligating the first oligonucleotide and the second oligonucleotide via the first single-stranded overhang of the first oligonucleotide and the single-stranded overhang of the second oligonucleotide, generating a first ligation product, whereby the first ligation product comprises a single-stranded overhang essentially corresponding to the second single-stranded overhang of the first oligonucleotide,

d) cutting the first ligation product with the first type II restriction enzyme thus releasing

- an elongated first at least partially double- stranded oligonucleotide having a first and a second single-stranded overhang, whereby the first single-stranded overhang is generated through the cutting of the restriction enzyme and whereby the second single-stranded overhang corresponds essentially to the second single-stranded overhang of the first at least partially double-stranded oligonucleotide, preferably the at least partially double-stranded oligonucleotide of step (a), and
- a truncated second at least partially double-stranded oligonucleotide;

e) immobilising the truncated second at least partially double stranded oligonucleotide of step d), the unreacted second at least partially double-stranded oligonucleotide and/or the uncut first ligation product via the modification to a surface;

f) optionally repeating steps a) to e), whereby the elongated first at least partially double-stranded oligonucleotide of step d) serves as the first at least partially double-stranded oligonucleotide in step a).

2. The method according to claim 1, comprising the following step

ca) immobilising the first ligation product via the long single-stranded overhang to a surface,

3. The method according to claim 2, wherein the surface comprises a nucleic acid having a single-stranded stretch which is at least partially complementary to the single-stranded overhang of the first ligation product.

4. The method according to any of claims 1 to 3, comprising the following step

cb) optionally washing the immobilised first elongation product; and

cc) releasing the immobilised first elongation product from the surface.

5. The method according to any of claims 1 to 4, wherein the length of the first single-stranded overhang of the first at least partially complementary oligonucleotide has a length of 1,2, 3, 4 or 5 nucleotides.

6. The method according to any of claims 1 to 5, wherein the second single-stranded overhang of the first oligonucleotide allows for a stable hybridisation to the single-stranded stretch of the nucleic acid comprised on the surface.

7. The method according to claim 6, wherein the hybridisation is stable under the reaction conditions of step cb).

8. The method according to any of claims 1 to 7, wherein the single-stranded overhang has a length from about 5 to 20 nucleotides, from about 10 to 20 nucleotides, from about 15 to 18 nucleotides, from about 5 to 10 nucleotides and from about 6 to 8 nucleotides, depending on the nature of the nucleotides.

9. The method according to any of claims 1 to 8, wherein the modification is a biotin modification.

10. The method according to any of claims 1 to 9, wherein the immobilisation of step e) occurs via interaction of the biotin and the surface, whereby the surface preferably comprises a biotin interaction group.

11. The method according to any of claims 1 to 10, wherein the biotin interaction group is selected from the group comprising avidine, streptavidine, extravidine, mutants of each thereof and synthetic biotin binding sites.

12. The method according to any of claims 1 to 11, wherein a part of the nucleic acid to be manufactured is part of the elongated first at least partially double-stranded oligonucleotide.

13. The method according to any of claims 1 to 12, wherein steps a) to e) are repeated at least once, whereby the nucleotides transferred from the second and any further at least partially double-stranded oligonucleotides provided in step b) to the first at least partially double-stranded oligonucleotides are the nucleic acid to be manufactured or a part thereof.

14. A method for the manufacture of a nucleic acid molecule, preferably a double-stranded nucleic acid molecule comprising the following steps:

a) providing an elongated first at least partially double-stranded oligonucleotide, preferably an elongated first at least partially double-stranded oligonucleotide obtainable by the method according to any of claims 1 to 13, whereby the nucleotides transferred from the second and/or any further at least partially double-stranded oligonucleotide are a first part of the nucleic acid molecule to be manufactured and the type IIS restriction enzyme is a first type IIS restriction enzyme,
b) providing another elongated first at least partially double-stranded oligonucleotide, preferably an elongated first at least partially double-stranded oligonucleotide obtainable by the method according to any of claims 1 to 13, whereby the nucleotides

transferred from the second and/or any further at least partially double-stranded oligonucleotide are a second part of the nucleic acid molecule to be manufactured and the type IIS restriction enzyme of the second at least partially double-stranded oligonucleotide is a second type IIS restriction enzyme and the type IIS restriction enzyme of the further at least partially double-stranded oligonucleotide is a further type IIS restriction enzyme, whereby the second type IIS restriction enzyme and/or the further type IIS restriction enzyme is different from the first type IIS restriction enzyme, and the second single-stranded overhang is different from the second single-stranded overhang of the elongated first at least partially double-stranded oligonucleotide as of step a)

c) ligating the oligonucleotides of step a) and b) producing a first extended ligation product,
d) immobilising the first extended ligation product of step c) via the second single-stranded overhang of the oligonucleotide of step a),
e) optionally washing away the supernatant,
f) releasing the first extended ligation product of step c),
g) immobilising the first extended ligation product of step c) via the second single-stranded overhang of the oligonucleotide of step b),
h) optionally washing away the supernatant,
i) releasing the first extended ligation product from step c),

whereby the first part of the nucleic acid molecule to be manufactured and the second part of the nucleic acid molecule to be manufactured are consecutively arranged in the nucleic acid molecule to be manufactured.

15. The method according to claim 14, wherein steps a) to i) are carried out in a separate reaction and whereby

- the elongated first at least partially double-stranded oligonucleotide of step a) comprises as nucleotides transferred from the second and/or any further at least partially double-stranded oligonucleotide nucleotides which form a third part of the nucleic acid molecule to be manufactured, and

- the another elongated first at least partially double-stranded oligonucleotide of step a) comprises as nucleotides transferred from the second and/or any further at least partially double-stranded oligonucleotide nucleotides which form a fourth part of the nucleic acid molecule to be manufactured,

generating a second extended ligation product.

16. The method according to claims 14 and 15,

wherein the first extended ligation product is cleaved by the second type IIS restriction enzyme generating

in step j)

- a cut first extended ligation product, and
- an at least partially double-stranded oligonucleotide corresponding to the first at least partially double-stranded oligonucleotide used in the generation of the elongated at least partially double-stranded oligonucleotide of step a),

wherein the second extended ligation product is cleaved by the first type IIS restriction enzyme generating

in step k)

- a cut second extended ligation product and
- an at least partially double-stranded oligonucleotide corresponding to the first at least partially double-stranded oligonucleotide used in the generation of the elongated at least partially double-stranded oligonucleotide of step b)

17. The method according to claims 16, comprising as step

l) ligating the cut first extended ligation product and the cut second extended ligation product.

18. The method according to claim 17, wherein the at least partially double-stranded oligonucleotide of step k) is immobilised through the second single-stranded overhang to a surface, whereby the surface comprises a nucleic acid molecule having at least a single-stranded stretch which is at least partially complementary to the second single-stranded overhang.

19. The method according to claim 17 and 18, wherein the at least partially double-stranded oligonucleotide of step l) is immobilised through the second single-stranded overhang to a surface, whereby the surface comprises a nucleic acid molecule having at least a single-stranded stretch which is at least partially complementary to the second single-stranded overhang.

20. The method according to any of claims 14 to 19, wherein the ligation product of step k) is used as an elongated first at least partially double stranded oligonucleotide in step a) and a ligation product obtained in step k) using parts 5 to 8 of the nucleic acid to be manufactured obtained by the method according to any of claims 14 to 19 is used as the another elongated first at least partially double-stranded oligonucleotide in step b).

21. The method according to any of claims 14 to 20, wherein the type IIS restriction enzyme is selected from the group comprising Eco 31I and Esp 3I.

22. A method for the manufacture of a nucleic acid molecule comprising the following steps:

a) providing a first ligation product, whereby the first ligation product consists of a first oligonucleotide moiety comprising a recognition site for a first type IIS restriction enzyme, a second oligonucleotide moiety comprising a recognition site for a second type IIS restriction enzyme and a modification allowing specific binding to a solid phase, and a third oligonucleotide moiety in between, whereby the third oligonucleotide moiety is a part of the nucleic acid molecule to be manufactured, and whereby the first and the second type IIS restriction enzymes

each generate upon cleavage an overhang, whereby preferably the overhang generated by the first type IIS restriction enzyme has a length which is different from the length of the overhang generated by the second type IIS restriction enzyme;

b) providing a second ligation product, whereby the second ligation product consists of a first oligonucleotide moiety comprising a recognition site for a third type IIS restriction enzyme, a second oligonucleotide moiety comprising a recognition site for a fourth type IIS restriction enzyme and a modification allowing specific binding to a solid phase, and a third oligonucleotide moiety in between, whereby the third oligonucleotide moiety is a part of the nucleic acid molecule to be manufactured, and whereby the third and the fourth type IIS restriction enzyme each generate upon cleavage an overhang, whereby optionally the overhang generated by the third type IIS restriction enzyme has a length which is different from the length of the overhang generated by the fourth type IIS restriction enzyme;

c) providing a third ligation product, whereby the third ligation product consists of a first oligonucleotide moiety comprising a recognition site for a fifth type IIS restriction enzyme, a second oligonucleotide moiety comprising a recognition site for a sixth type IIS restriction enzyme and a modification allowing specific binding to a solid phase, and a third oligonucleotide moiety in between, whereby the third oligonucleotide moiety is a part of the nucleic acid molecule to be manufactured, and whereby the fifth and the sixth type IIS restriction enzymes each generate an overhang, whereby optionally the overhang generated by the fifth type IIS restriction enzyme has a length which is different from the length of the overhang generated by the sixth type IIS restriction enzyme;

d) providing a fourth ligation product, whereby the fourth ligation product consists of a first oligonucleotide moiety comprising a recognition site for a seventh type IIS restriction enzyme, a second oligonucleotide moiety comprising a recognition site for an eighth type IIS restriction enzyme and a modification allowing specific binding to a solid phase, and a third oligonucleotide moiety in between, whereby

the third oligonucleotide moiety is a part of the nucleic acid molecule to be manufactured, and whereby the seventh and the eighth type IIS restriction enzyme each generate an overhang, whereby preferably the overhang generated by the seventh type IIS restriction enzyme has a length which is different from the length of the overhang generated by the eighth type IIS restriction enzyme;

whereby the third oligonucleotide moiety of the first and of the second ligation product each comprise the part of the nucleic acid to be manufactured in the same orientation and the third oligonucleotide moiety of the third and of the fourth ligation product each comprise the part of the nucleic acid to be manufactured in the same orientation,

whereby

either the orientation of the parts of the nucleic acid molecule to be manufactured as contained in the first and second ligation product is the same as in the nucleic acid molecule to be manufactured and the orientation of the parts of the nucleic acid molecule to be manufactured as contained in the third and the fourth ligation product is opposite to the orientation as in the nucleic acid molecule to be manufactured;

or the orientation of the parts of the nucleic acid molecule to be manufactured as contained in the first and second ligation product is opposite to the orientation as in the nucleic acid molecule to be manufactured, and the orientation of the parts of the nucleic acid molecule to be manufactured as contained in the third and the fourth ligation product is the same as in the nucleic acid molecule to be manufactured;

e) cutting the first ligation product with the first type IIS restriction enzyme generating a first cut ligation product and cutting the second ligation product with the fourth restriction enzyme generating a second cut ligation product;

f) cutting the third ligation product with the sixth type IIS restriction enzyme generating a third cut ligation product and cutting the fourth ligation product with the seventh restriction enzyme generating a fourth cut ligation product;

g) combining and ligating the first cut ligation product and the second cut ligation product generating a first transposition product, whereby the first transposition product is cut by the third type II S restriction enzyme generating a cut first transposition product;

h) combining and ligating the third ligation product and the fourth ligation product providing a second transposition product, whereby the second transposition product is cut by both the fifth and the eighth type IIS restriction enzyme generating a cut second transposition product; and

i) ligating the cut first transposition product and the cut second transposition product generating a second order transposition product.

23. The method according to claim 22, wherein the first type IIS restriction enzyme, the third type IIS restriction enzyme, the fifth type IIS restriction enzyme and the seventh type IIS restriction enzyme are the same.

24. Method according to claim 22 or 23, wherein the second type IIS restriction enzyme and the eighth type IIS restriction enzyme are the same.

25. Method according to any of the claims 22 to 24, wherein the fourth type IIS restriction enzyme and the sixth type II S restriction enzyme are the same

26. The method according to any of claims 22 to 25, wherein the restriction enzyme is selected from the group comprising Esp3I, Eco31I and Eam 1104 I.

27. The method according to any of claims 22 to 26, wherein the second and the third ligation product are immobilized to a surface via the modification prior to the cutting with the restriction enzymes.

28. The method according to claim 28, wherein the supernatant obtained by cutting the immobilized second ligation product is ligated to the immobilized cut first ligation product, and wherein the supernatant obtained by cutting the immobilized third ligation product is ligated to the immobilized cut fourth ligation product.

29. The method according to any of claims 22 to 29, wherein the length of the double-stranded stretch of the second cut transposition product is selected from the group comprising 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37 or 38 base pairs.

30. The method according to any of claims 22 to 30, wherein the third oligonucleotide moiety of the first ligation product forms a first part of the nucleic acid molecule to be manufactured and the third oligonucleotide moiety of the second ligation product forms a second part of the nucleic acid molecule to be manufactured, whereby the first part and the second part are consecutive parts of the nucleic acid molecule to be manufactured and both the first part and the second part have the same orientation in the first ligation product and the second ligation product.

31. The method according to any of claims 22 to 31, wherein the third oligonucleotide moiety of the third ligation product forms a third part of the nucleic acid molecule to be manufactured and the third oligonucleotide moiety of the fourth ligation product forms a fourth part of the nucleic acid molecule to be manufactured, whereby the third part and the fourth part are consecutive parts of the nucleic acid molecule to be manufactured and both the third part and the fourth part have the same orientation in the third ligation product and the fourth ligation product, whereby the orientation of the third part and the fourth part is inverse compared to the orientation of the first and second part.

32. The method according to any of claims 22 to 32, wherein the second order transposition product is used as the first transposition product in step f) of the method, and a further second order transposition product is used as the second transposition product of step g) of the method, whereby the parts of the nucleic acid to be manufactured provided by the second order transposition product and the parts of the nucleic acid to be manufactured provided by the further second order transposition product are in an inverse orientation

33. A method for the manufacture of a nucleic acid molecule comprising the following steps:

a) providing a first ligation product, whereby the first ligation product consists of a first oligonucleotide moiety comprising a recognition site for a first type IIS restriction enzyme, a second oligonucleotide moiety comprising a recognition site for a second type IIS restriction enzyme and a modification allowing specific binding to a solid phase, and a third oligonucleotide moiety in between, whereby the third oligonucleotide moiety is a part of the nucleic acid molecule to be manufactured, and whereby the first and the second type IIS restriction enzymes each generate upon cleavage an overhang, whereby preferably the overhang generated by the first type IIS restriction enzyme has a length which is different from the length of the overhang generated by the second type IIS restriction enzyme;

b) providing a second ligation product, whereby the second ligation product consists of a first oligonucleotide moiety comprising a recognition site for a third type IIS restriction enzyme, a second oligonucleotide moiety comprising a recognition site for a fourth type IIS restriction enzyme and a modification allowing specific binding to a solid phase, and a third oligonucleotide moiety in between, whereby the third oligonucleotide moiety is a part of the nucleic acid molecule to be manufactured, and whereby the third and the fourth type IIS restriction enzyme each generate upon cleavage an overhang, whereby optionally the overhang generated by the third type IIS restriction enzyme has a length which is different from the length of the overhang generated by the fourth type IIS restriction enzyme;

c) providing a third ligation product, whereby the third ligation product consists of a first oligonucleotide moiety comprising a recognition site for a fifth type IIS restriction enzyme, a second oligonucleotide moiety comprising a recognition site for a sixth type IIS restriction enzyme and a modification allowing specific binding to a solid phase, and a third oligonucleotide moiety in between, whereby the third oligonucleotide moiety is a part of the nucleic acid molecule to be manufactured, and whereby the fifth and the sixth type IIS restriction enzymes

each generate an overhang, whereby optionally the overhang generated by the fifth type IIS restriction enzyme has a length which is different from the length of the overhang generated by the sixth type IIS restriction enzyme;

d) providing a fourth ligation product, whereby the fourth ligation product consists of a first oligonucleotide moiety comprising a recognition site for a seventh type IIS restriction enzyme, a second oligonucleotide moiety comprising a recognition site for an eighth type IIS restriction enzyme and a modification allowing specific binding to a solid phase, and a third oligonucleotide moiety in between, whereby the third oligonucleotide moiety is a part of the nucleic acid molecule to be manufactured, and whereby the seventh and the eighth type IIS restriction enzyme each generate an overhang, whereby preferably the overhang generated by the seventh type IIS restriction enzyme has a length which is different from the length of the overhang generated by the eighth type IIS restriction enzyme;

e) cutting the first ligation product with the second type IIS restriction enzyme generating a first cut ligation product and cutting the fourth ligation product with the fourth restriction enzyme generating a fourth cut ligation product;

f) cutting the second ligation product with the second type IIS restriction enzyme generating a second cut ligation product and cutting the third ligation product with the sixth restriction enzyme generating a third cut ligation product;

g) combining the first cut ligation product and the second cut ligation product in the presence of a ligase and the third type IIS restriction enzyme generating a first transposition product, generating a cut first transposition product;

h) combining the third ligation product and the fourth ligation product in the presence of a ligase and the fifth type IIS restriction enzyme providing a second transposition product, whereby the second transposition product is cut by the fifth and the eighth type IIS restriction enzyme generating a cut second transposition product; and

i) ligating the cut first transposition product and the cut second transposition product generating a second order transposition product.

34. The method according to claim 33, wherein the first type IIS restriction enzyme, the third type IIS restriction enzyme, the fifth type IIS restriction enzyme, and the seventh type IIS restriction enzyme are the same.

35. Method according to claim 33 or 34, wherein the second type IIS restriction enzyme and the eighth type IIS restriction enzyme are the same.

36. Method according to any of the claims 33 to 35, wherein the fourth type IIS restriction enzyme and the sixth type II S restriction enzyme are the same.

37. The method according to any of claims 33 to 36, wherein the restriction enzyme is selected from the group comprising Esp3I, Eco31 and Eam 1104 I.

38. The method according to any of claims 33 to 37, wherein the first and the fourth ligation product are immobilized to a surface via the modification prior to the cutting with the restriction enzymes.

39. The method according to claim 38, wherein the supernatant obtained by cutting the immobilized second ligation product is ligated to the immobilized cut first ligation product, while the third type IIS restriction enzyme is present and, preferably active, and wherein the supernatant obtained by cutting the immobilized third ligation product is ligated to the immobilized cut fourth ligation product, while the fifth type IIS restriction enzyme is present.

40. The method according to any of claims 33 to 39, wherein the length of the double-stranded stretch of the second cut transposition product is selected from the group comprising 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37 or 38 base pairs.

41. The method according to any of claims 33 to 40, whereby the third oligonucleotide moiety of the first and of the third ligation product each comprise the part of the nucleic acid to be manufactured in the same orientation and the third oligonucleotide moiety of the second and

of the fourth ligation product each comprise the part of the nucleic acid to be manufactured in the same orientation,

whereby

either the orientation of the parts of the nucleic acid molecule to be manufactured as contained in the first and third ligation product is the same as in the nucleic acid molecule to be manufactured and the orientation of the parts of the nucleic acid molecule to be manufactured as contained in the second and the fourth ligation product is opposite to the orientation as in the nucleic acid molecule to be manufactured;

or the orientation of the parts of the nucleic acid molecule to be manufactured as contained in the first and third ligation product is opposite to the orientation as in the nucleic acid molecule to be manufactured, and the orientation of the parts of the nucleic acid molecule to be manufactured as contained in the second and the fourth ligation product is the same as in the nucleic acid molecule to be manufactured.

42. The method according to any of claims 33 to 41, wherein the second order transposition product is used as the first transposition product in step f) of the method, and a further second order transposition product is used as the second transposition product of step g) of the method, whereby the parts of the nucleic acid to be manufactured provided by the second order transposition product and the parts of the nucleic acid to be manufactured provided by the further second order transposition product are in an inverse orientation

43. A method for the ligation of a first oligonucleotide and a second oligonucleotide, whereby

- the first oligonucleotide and the second oligonucleotide are contained in a ligation reaction;
- the first oligonucleotide is an at least partially double-stranded oligonucleotide having a single stranded overhang, and the second oligonucleotide is an at least partially double-stranded oligonucleotide having a single stranded overhang, whereby the single-stranded overhang of the first oligonucleotide and the sinlge-

stranded overhang of the second oligonucleotide are overlapping so as to allow for a ligation of the first and the second oligonucleotide;

- the ligation reaction comprises at least a further oligonucleotide, whereby such further oligonucleotide comprises a partial sequence, whereby the partial sequence is suitable to be ligated to the first oligonucleotide or to the second oligonucleotide;
- and a capping oligonucleotide, whereby such capping oligonucleotide comprises an at least partially a double-stranded structure and a single-stranded overhang, whereby the single stranded overhang allows ligation to the further oligonucleotide.

44. The method according to claim 43, wherein the single-stranded overhang of the first oligonucleotide and the single-stranded overhang of the second oligonucleotide are complementary to each other, preferably allowing a perfect match.

45. The method according to any of claims 43 and 44, wherein the length of the single-stranded overhang of the first oligonucleotide and of the second oligonucleotide is each independently selected from the group comprising a two nucleotide overhang, a three nucleotide overhang, a four nucleotide overhang, a five nucleotide overhang, a six nucleotide overhang and a seven nucleotide overhang.

46. The method according to any of claims 43 to 45, wherein the partial sequence of the further oligonucleotide is essentially complementary to the single-stranded overhang, or part thereof, of the first oligonucleotide or of the second oligonucleotide.

47. The method according to any of claims 43 to 46, wherein the partial sequence of the further oligonucleotide, or part thereof, is complementary to a part of the single-stranded overhang, or part thereof, of the first oligonucleotide or of the second oligonucleotide.

48. The method according to any of claims 43 to 47, wherein the further oligonucleotide is at least partially double-stranded and comprises a single-stranded overhang, whereby the single-stranded overhang comprises the partial sequence.

49. The method according to any of claims 43 to 48, wherein the single-stranded overhang of the capping oligonucleotide, or part thereof, is essentially complementary to the partial sequence of the further oligonucleotide, or part thereof.

50. The method according to any of claims 43 to 49, wherein the ligation reaction between the further oligonucleotide and the capping oligonucleotide is preferred to the ligation of the further oligonucleotide and the first and second oligonucleotide, respectively.

51. The method according to any of claims 43 to 50, wherein the capping oligonucleotide is not ligating to the first and second oligonucleotide in the ligation reaction.

52. The method according to any of claims 43 to 51, wherein the capping oligonucleotide is contained in the ligation reaction in excess, preferably 2-10fold.

53. The method according to any of claims 43 to 52, wherein the capping oligonucleotide comprises a loop structure, preferably a loop structure at the end opposite to the single-stranded overhang.

54. Method for the manufacture of a nucleic acid molecule, comprising the steps of

a) providing a first at least partially double-stranded oligonucleotide which comprises a recognition site for a first type IIS restriction enzyme which cuts outside its recognition site, and which oligonucleotide comprises a single-stranded overhang;

b) providing a second at least partially double-stranded oligonucleotide which comprises a modification allowing the oligonucleotide to be coupled to a surface, whereby the oligonucleotide further comprises a recognition site or a part thereof or a sequence which is complementary thereto, for a second type IIS restriction enzyme which cuts outside its recognition site, and which second oligonucleotide comprises a single-stranded overhang;

c) ligating the first and the second oligonucleotide via their overhangs generating a first ligation product;

d) immobilising the first ligation product on a surface via the modification contributed by the second oligonucleotide

e) cutting the immobilised first ligation product with the second type IIS restriction enzyme thus releasing an elongated first oligonucleotide having an overhang and a shortened second oligonucleotide, which remains bound to the surface;

f) providing a further at least partially double-stranded oligonucleotide which has a modification allowing the further oligonucleotide to be specifically coupled to a surface, whereby the oligonucleotide contains a recognition site for a second or a further type IIS restriction enzyme and a single-stranded overhang which is complementary to the overhang of the elongated first oligonucleotide;

g) ligating the further at least partially double-stranded oligonucleotide with the elongated first oligonucleotide via their overhangs generating a second level ligation product;

h) cutting the second level ligation product with the second or further type IIS restriction enzyme thus generating a second level elongated oligonucleotide having an overhang and a shortened further oligonucleotide;

i) immobilising the shortened further oligonucleotide;

j) repeating steps f) to i) at least once, generating in step g) a higher level ligation product, whereby in the last repetition the incoming further oligonucleotide comprises a recognition site for a type IIS restriction enzyme which upon cleavage produces a single-stranded overhang identical in length to the overhang generated by the first type IIS restriction enzyme specific for the first oligonucleotide, and steps h) and i) are replaced by the steps k) and l)

k) immobilising the higher level ligation product via the modification provided by the further oligonucleotide; and

l) cutting the higher level ligation product with the further type IIS restriction enzyme, leaving the part of the nucleic acid to be manufactured attached to the first oligonucleotide, which is preferably released into the supernatant, and more preferably allowing its transfer to a new reaction vessel.

55. Method for the manufacture of a nucleic acid molecule, comprising the steps of

a) providing a first at least partially double-stranded oligonucleotide which comprises a recognition site for a first type IIS restriction enzyme which cuts outside its recognition site, and which oligonucleotide comprises a single-stranded overhang;

b) providing a second at least partially double-stranded oligonucleotide which comprises a modification allowing the oligonucleotide to be coupled to a surface, whereby the oligonucleotide further comprises a recognition site or a part thereof or a sequence which is complementary thereto, for a second type IIS restriction enzyme which cuts outside its recognition site, and which second oligonucleotide comprises a single-stranded overhang;

c) ligating the first and the second oligonucleotide via their overhangs generating a first ligation product;

d) immobilising the first ligation product on a surface via the modification contributed by the second oligonucleotide;

e) cutting the immobilised first ligation product with the second type IIS restriction enzyme thus releasing an elongated first oligonucleotide having an overhang and a shortened second oligonucleotide, which remains bound to the surface;

f) providing a further at least partially double-stranded oligonucleotide which has a modification allowing the further oligonucleotide to be specifically coupled to a surface, whereby the oligonucleotide contains a recognition site for a second or further type IIS restriction enzyme and a single-stranded overhang which is complementary to the overhang of the elongated first oligonucleotide;

g) ligating the further at least partially double-stranded oligonucleotide with the elongated first oligonucleotide via their overhangs generating a second level ligation product;

h) cutting the second level ligation product with the second or further type IIS restriction enzyme thus generating a second level elongated oligonucleotide having an overhang and a shortened further oligonucleotide;

i) immobilising the shortened further oligonucleotide;

j) repeating steps f) to i) at least once, generating in step g) a higher level ligation product, whereby in the last repetition the incoming further oligonucleotide

comprises a recognition site for a type IIS_restriction enzyme which upon cleavage produces a single-stranded overhang identical in length to the overhang generated by the first type IIS restriction_enzyme specific for the first oligonucleotide, and steps h) and i) are replaced by the steps k) and l)

k) immobilising the higher level ligation product via the modification provided by the further oligonucleotide; and

l) cutting the immobilised higher level ligation product with the type IIS restriction enzyme specific for the first oligonucleotide, leaving the part of the nucleic acid to be manufactured attached to the further oligonucleotide, which is immobilised on a surface.

56. The method according to claim 54 and/or 55, wherein as step m) the cut immobilised higher level ligation product of step l) of claim 55 is ligated with the cut higher level ligation product of step l) of claim 54.

57. The method according to claim 56, wherein the cut higher level ligation product of step l) of claim 56 is cleaved with the second type IIS restriction enzyme prior to the ligation step m).

58. The method according to any of claims 54 to 57, wherein the number of repetitions in step j) is two, three, four, five or six

59. The method according to any of claims 54 to 58, wherein the overhang is a 5' or a 3' overhang.

60. The method according to any of claims 54 to 59, wherein the overhang is selected from the group comprising a one nucleotide overhang, a two nucleotides overhang, a three nucleotides overhang, a four nucleotides overhang, and a five nucleotides overhang.

61. The method according to any of claims 54 to 60, wherein the at least partially double-stranded oligonucleotide comprises a constant region and a variable region, whereby the constant region contains the recognition site for a type IIS restriction enzyme, and the variable region contains a nucleic acid sequence which corresponds to a part of the nucleic acid sequence of the nucleic acid molecule to be manufactured.

62. The method according to any of claims 54 to 61, wherein the further type IIS restriction enzyme is the second type IIS restriction enzyme.

63. The method according to any of claims 54 to 62, wherein the elongated oligonucleotide is transferred to a different reaction vessel.

64. The method according to any of claims 54 to 63, wherein the second level elongated oligonucleotide is transferred to a different reaction vessel.

65. The method according to any of claims 54 to 64, wherein the second level elongated oligonucleotide is used as the elongated oligonucleotide in step g).

66. The method according to any of claims 54 to 65, wherein the modification of the elongated oligonucleotide and/or of the higher level ligation product is provided by the further at least partially double-stranded oligonucleotide.

67. Use of the method according to any of claims 43 to 53 in a method according to any of the preceding claims, more particularly in any ligation reaction thereof.